

苦皮藤素 V 对东方粘虫肌细胞的影响

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摘要: 苦皮藤素 V 是从杀虫植物苦皮藤 *Celastrus angulatus* Max. 根皮中分离的一种对昆虫具毒杀活性的新化合物。采用电子显微镜技术研究了苦皮藤素 V 对东方粘虫 *Mythimna separata* (Walker) 肌肉系统的作用。电镜观察发现, 苦皮藤素 V 对东方粘虫成虫飞行肌和幼虫体壁肌均具致毒作用, 中毒试虫肌细胞特别是肌细胞的质膜及内膜系统发生明显病变: 肌膜破坏, 脱落; 线粒体肿胀, 空泡化, 崩解; 肌原纤维与线粒体间隙增大; 肌质网扩张, 产生髓鞘样结构; 细胞核肿胀, 核质浓缩, 核膜破坏; 微气管与肌细胞之间间隙增大; 肌小节弥散、排列紊乱。这些结果表明, 肌细胞质膜及内膜系统可能是苦皮藤素 V 的一个作用部位。

关键词: 苦皮藤素 V; 东方粘虫; 肌细胞毒性

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Effects of celangulin V on muscle cells of *Mythimna separata*

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Abstract: Celangulin V isolated from the root bark of *Celastrus angulatus* Max. is a new kind of insecticidal compound. The effects of celangulin V on the muscle cells of *Mythimna separata* were determined with electron microscopy observation. It was found that celangulin V had a myotoxic effect on the adult flight muscles and larval skeletal muscles of *M. separata*. The sarcolemma and inner membrane were disrupted and mitochondria became swollen, vacuolated and disintegrated. The space between myofibrils and mitochondria significantly increased, the sarcoplasmic reticulum spread and pith tissue occurred, the nucleus became swollen, nucleoplasm concentrated and the nuclear membrane disrupted, the space around trachea enlarged and sarcomeres contracted abruptly. These results suggest that the sarcolemma and inner membrane might be the target acted on by celangulin V.

Key words: celangulin V; *Mythimna separata*; myotoxicity

苦皮藤素 V 是从杀虫植物苦皮藤 *Celastrus angulatus* Max. 根皮中分离的一种对昆虫具毒杀活性的二氢沉香呋喃类化合物, 试虫摄食苦皮藤素 V 后, 先是表现兴奋、抽搐、痉挛, 继而呕吐泄泻, 体液大量流失而致死 (吴文君等, 1994)。电镜观察表明, 苦皮藤素 V 破坏粘虫中肠肠壁细胞, 特别是肠壁细胞的质膜和内膜系统, 引起体液大量流失 (刘惠霞等, 1998)。神经电生理和生化分析结果表明: 苦皮藤素 V 不影响试虫中枢神经系统轴突和突触的兴奋性传导, 但可引起试虫神经-肌肉接点兴奋性递质 Glu 含量升高, 抑制性递质 γ -氨基丁酸 (GABA) 含量降低, 二者协同作用可能导致试虫兴

奋、抽搐 (杨润亚等, 2000)。但苦皮藤素 V 对肌肉这个神经支配的运动器官的作用, 尚未见相关的研究报道。我们以东方粘虫 *Mythimna separata* 为试虫, 观察苦皮藤素 V 对成虫飞行肌及幼虫体壁肌的作用, 以期进一步阐明苦皮藤素 V 的作用部位及引起试虫兴奋、抽搐和痉挛的机理。

1 材料与方法

1.1 材料

以室内饲养的东方粘虫幼虫和成虫为试虫。从苦皮藤根皮中提取分离的苦皮藤素 V (2 β , 8 α -乙酰

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氧基-9 β -苯甲酰氧基-1 β ,12-异丁酰氧基-4 α ,6 α -羟基- β -二氢沉香呋喃),纯度 95%,配成 100 mg/mL 的丙酮溶液供试。

1.2 方法

1.2.1 试虫处理:挑选羽化后第 4 天的粘虫雌蛾,从前胸背板肌肉注射 1 μ L 100 mg/mL 苦皮藤素 V 的丙酮溶液,对照注射 1 μ L 丙酮,12 h 后取胸部背纵肌制备样品。

挑选蜕皮后第 2 天的 6 龄幼虫,饥饿 24 h。处理组试虫饲喂载毒叶碟(在 0.5 cm \times 0.5 cm 小麦叶片上均匀点涂 1 μ L 100 mg/mL 苦皮藤素 V 的丙酮溶液,溶剂挥发后喂虫,一虫一叶),对照组试虫饲喂同样大小点涂 1 μ L 丙酮的叶片。分别于处理组试虫出现兴奋、抽搐和脱水症状时取腹部体壁肌制备样品。

1.2.2 肌肉样品的处理:成虫飞行肌取中胸背纵肌(罗礼智,1996),幼虫取腹部第 2~6 节间体壁肌。低温下用 4% 戊二醛前固定,1% 锇酸后固定,组织经系列丙酮(50% \rightarrow 70% \rightarrow 80% \rightarrow 90% \rightarrow 100%)脱水之后,用 Epon812 包埋剂浸透包埋,35 $^{\circ}$ C、45 $^{\circ}$ C 和 60 $^{\circ}$ C 各聚合 1 天, LKBV 型超薄切片机切片,再以醋酸铀和柠檬酸铅双重染色。

1.2.3 电镜观察:用 JEM-2000EX 透射电镜观察肌细胞并拍照,加速电压为 80 kV。

2 结果与分析

2.1 苦皮藤素 V 对粘虫成虫飞行肌的作用

电镜观察表明,对照组成虫飞行肌细胞肌膜完好,肌小节、肌原纤维横纹、线粒体排列规则整齐,线粒体和肌原纤维接触紧密,核膜完整、核物质分布均一(图版 I:1)。苦皮藤素 V 处理后,粘虫成虫飞行肌肌膜局部损伤脱落,暴露出肌原纤维丝(图版 I:5);线粒体皱缩、崩解、出现致密颗粒(图版 I:4);肌原纤维和线粒体之间间隙增大(图版 I:6);核质浓缩,核膜破坏(图版 I:2)。

2.2 苦皮藤素 V 对粘虫幼虫体壁肌的作用

电镜观察表明,对照组幼虫体壁肌细胞结构完好,肌膜完整;肌原纤维排列规则有序,Z-线致密清晰;核膜完整、核物质分布均一(图版 I:7);肌质网结构完好致密(图版 II:1);气管与肌细胞紧密接触(图版 II:2);线粒体内膜、外膜和嵴清晰可见(图版 II:3)。粘虫取食苦皮藤素 V 后表现兴奋、抽搐及体液流失的不同时期,随着中毒程度

的加深,肌细胞呈进行性病变。幼虫中毒兴奋期,肌膜局部轻度损伤(图版 I:8);抽搐期,肌膜部分断裂、脱落,并出现髓鞘样结构(图版 I:9);至幼虫中毒最严重的脱水期,肌膜严重损伤脱落(图版 I:3)。从兴奋期开始至抽搐、脱水期,肌质网逐级进行性扩张(图版 II:4~6);肌原纤维 Z-线弥散呈间断点状排列,继而导致肌节排列紊乱(图版 II:7~9);气管与肌细胞之间间隙呈进行性增大(图版 II:10~12);核明显肿胀、核质浓缩,且至脱水期核膜破坏(图版 III:1~3)。线粒体肿胀、出现空白亮区,嵴紊乱、空泡化、继而模糊不清(图版 III:4~6)。

上述结果表明,苦皮藤素 V 可以直接作用于粘虫成虫的飞行肌和幼虫的体壁肌组织,对肌细胞、特别是肌细胞的质膜及内膜(线粒体膜、内质网膜和核膜)系统造成破坏,致使细胞膜系统丧失固有的屏障作用和正常的渗透平衡功能,从而导致内质网扩张,线粒体肿胀、嵴模糊不清及细胞核肿胀、核质浓缩等细胞器病变;并对肌肉的收缩机构——肌原纤维造成一定破坏而影响肌肉收缩;同时还通过影响肌细胞与微气管之间的连接而影响肌细胞的氧气供应。由此推测,肌细胞可能是苦皮藤素 V 的又一个作用部位。

3 讨论

已有的研究认为,苦皮藤素 V 作为一种胃毒剂,可直接作用于粘虫中肠细胞的质膜及内膜系统,致使肠壁穿孔,血淋巴大量涌入消化道,试虫因呕吐泄泻、体液大量流失而致死(刘惠霞等,1998;杨润亚等,2001)。本研究结果表明:苦皮藤素 V 不仅作用于中肠细胞的质膜及内膜系统,也可作用于肌细胞的质膜及内膜系统,如引起线粒体膜、肌质网膜和核膜等不同程度地瓦解和破坏。这说明肌细胞质膜及内膜系统可能是苦皮藤素 V 的又一个作用部位。昆虫死亡实质上是指细胞生命活动的结束即细胞死亡。在多细胞生物中,细胞死亡有两种不同的形式,一种是坏死性或意外性死亡(necrosis or accidental cell death),它是由于某些外界因素,如高热、物理、化学损伤和生物的侵袭等,造成细胞急速死亡而结束其生命;另一种是细胞自然凋亡(翟中和等,1994)。苦皮藤素 V 作为化学毒物可直接造成中肠肠壁细胞、肌细胞等坏死性死亡。同时,由于昆虫循环系统属开放式循环,所有

内部器官都浸浴在血液中(王荫长, 1994), 血液的大量流失又可能会使其它组织如脂肪体、神经等组织细胞因缺乏营养而坏死, 最后导致试虫死亡。苦皮藤素 V 对这些组织细胞的作用, 有待进一步研究。

已有的研究还认为, 苦皮藤素 V 处理后, 粘虫所表现出的兴奋、抽搐症状, 是由于神经-肌肉接点兴奋性神经递质 Glu 含量升高, 抑制性神经递质 γ -氨基丁酸含量降低所致(杨润亚等, 2000)。本研究推测幼虫表现的兴奋、抽搐症状, 也可能与苦皮藤素 V 处理后, 肌细胞的损伤有关。在苦皮藤素 V 作用下, 由于肌质网膜破坏, 大量 Ca^{2+} 从肌质网释放, 使胞浆 Ca^{2+} 浓度升高, 可能产生短暂的肌源性肌肉收缩; 但随着肌细胞破坏程度的加重, 微气管与肌细胞之间间隙扩大, 使肌细胞氧气供应受阻; 同时, 线粒体肿胀、瓦解直接影响肌细胞的能量供应; 肌膜破坏导致肌细胞兴奋传导受阻, 试虫兴奋性逐渐降低; 随后, 肌肉组织瓦解, 丧失收缩能力, 试虫进入麻痹状态。关于肌细胞 Ca^{2+} 浓度的变化也有待于深入探讨。

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图 版 说 明 (Explanation of Plates)

图版 I Plate I

1. 对照组粘虫成虫飞行肌细胞膜(M)完好, 肌原纤维(MF)横纹、肌小节排列规则, 线粒体(Mt)和肌原纤维接触紧密, 核膜(NM)完整、核物质分布均一 Untreated adult showing intact cell membrane (M), horizontal grain of myofibrils (MF) and regularly arranged sarcomeres, mitochondria (Mt) attached closely to myofibrils, undamaged nuclear membrane, nuclear cytoplasm well-distributed in flight muscle (10 000 \times);
2. 处理组粘虫成虫飞行肌细胞核核质浓缩、核膜破坏 Treated adult showing concentrated nucleoplasm and disrupted nuclear membrane in flight muscle cells (10 000 \times);
3. 处理组粘虫幼虫中毒失水期体壁肌膜严重损伤脱落 Treated larvae showing seriously disrupted muscular membrane in skeletal muscle cells when larvae were losing body fluid (10 000 \times);
4. 处理组粘虫成虫飞行肌线粒体(Mt)崩解、出现致密颗粒 Treated adult showing mitochondria disintegration and electron dense particles in flight muscles (40 000 \times);
5. 处理组粘虫成虫飞行肌肌膜局部损伤脱落、暴露出肌原纤维丝 Treated adult showing muscular membrane disrupted locally and exposed myoneme in flight muscles (10 000 \times);
6. 处理组粘虫成虫飞行肌肌原纤维和线粒体之间间隙增大 Treated adult showing the enlarged space between myofibril and mitochondria in flight muscles (10 000 \times);
7. 对照组粘虫幼虫体壁肌细胞结构完好, 质膜、核膜完整、核物质分布均一; 肌原纤维排列规则有序、Z-线致密清晰 Untreated larvae showing intact cell composition, plasma membrane and nuclear membrane, well-distributed nucleoplasm; regularly arranged myofibrils, compact and distinct Z-line (7 500 \times);
8. 处理组粘虫幼虫中毒兴奋期体壁肌膜局部轻度损伤 Treated larvae showing muscular membrane disrupted slightly in skeletal muscles when larvae become excited (20 000 \times);
9. 处理组粘虫幼虫中毒抽搐期体壁肌膜部分断裂脱落, 出现髓鞘样结构 Treated larvae showing disintegrated muscular membrane and partly de-

tached and medullary sheath structure (MS) in skeletal muscles (20 000 \times).

M: 肌膜 muscular membrane; MF: 肌原纤维 myofibril; Mt: 线粒体 mitochondria; N: 细胞核 nucleus; NM: 核膜 nuclear membrane; BM: 底膜 basilar membrane; MS: 髓鞘样结构 medullary sheath structure; T: 微气管 tracheole; SR: 肌质网 sarcoplasmic reticulum; Z: Z-线 Z-line. 下同 The same for the following plates.

图版 II Plate II

1. 对照组粘虫幼虫体壁肌细胞肌质网 (SR) 结构完好 Untreated larvae showing structure of sarcoplasmic reticulum (SR) was undamaged in skeletal muscles (7 500 \times);
2. 对照组粘虫幼虫体壁肌微气管与肌细胞接触紧密 Untreated larvae showing tracheole attached closely to muscle cell (10 000 \times);
3. 对照组粘虫幼虫体壁肌细胞线粒体内膜、外膜、嵴清晰可见 Untreated larvae showing distinct mitochondrial inner membrane, outer membrane and cristae in skeletal muscle cells (30 000 \times);
4. 处理组粘虫幼虫中毒兴奋期体壁肌细胞肌质网扩张 Treated larvae showing expanded sarcoplasmic reticulum in skeletal muscle cells when larvae became excited (7 500 \times);
5. 处理组粘虫幼虫中毒抽搐期体壁肌细胞肌质网进一步扩张 Treated larvae showing much expanded sarcoplasmic reticulum in skeletal muscle cells when larvae became convulsive (7 500 \times);
6. 处理组粘虫幼虫中毒失水期体壁肌细胞肌质网进一步扩张 Treated larvae showing much expanded sarcoplasmic reticulum in skeletal muscle cells when larvae began to lose body fluid (7 500 \times);
7. 处理组粘虫幼虫中毒兴奋期体壁肌细胞肌原纤维 Z-线呈间断点状排列 Treated larvae showing Z-line ranged in a dotted line in myofibril of skeletal muscle cells when larvae became excited (20 000 \times);
8. 处理组粘虫幼虫中毒抽搐期体壁肌细胞肌原纤维 Z-线弥散、变淡、肌节排列紊乱 Treated larvae showing dispersed Z-line ranged, disordered sarcomeres in myofibril of skeletal muscle cell when larvae became convulsive (20 000 \times);
9. 处理组粘虫幼虫中毒失水期体壁肌细胞肌原纤维 Z-线弥散、变淡、肌节排列紊乱 Treated larvae showing dispersed Z-line, disordered sarcomeres in myofibril of skeletal muscle cell when larvae began to lose body fluid (20 000 \times);
10. 处理组粘虫幼虫中毒兴奋期体壁肌微气管与肌细胞之间出现间隙 Treated larvae showing the gap between tracheole and muscle cell in the skeletal muscle when larvae became excited (10 000 \times);
11. 处理组粘虫幼虫中毒抽搐期体壁肌微气管与肌细胞之间间隙增大 Treated larvae showing enlarged gap between tracheole and muscle cell in skeletal muscle when larvae became convulsive (10 000 \times);
12. 处理组粘虫幼虫中毒失水期体壁肌微气管与肌细胞之间间隙更大 Treated larvae showing the larger gap between tracheole and muscle cell in skeletal muscle when larvae began to lose body fluid (10 000 \times).

图版 III Plate III

1. 处理组粘虫幼虫中毒兴奋期体壁肌细胞核 (N) 肿胀、核质浓缩 Treated larvae showing swollen nucleus, nucleoplasm concentrated in skeletal muscles when the larvae became excited (4 000 \times);
2. 处理组粘虫幼虫中毒抽搐期体壁肌细胞核严重肿胀、核质浓缩 Treated larvae showing grossly swollen nucleus, nucleoplasm concentrated in skeletal muscles when larvae became convulsive (4 000 \times);
3. 处理组粘虫幼虫中毒失水期体壁肌细胞核严重肿胀、核质浓缩、核膜破坏 Treated larvae showing grossly swollen nucleus, concentrated nucleoplasm and nuclear membrane disrupted in skeletal muscles when larvae began to lose body fluid (4 000 \times);
4. 处理组粘虫幼虫中毒兴奋期体壁肌细胞线粒体 (Mt) 肿胀, 出现空白亮区 Treated larvae showing swollen mitochondria and bright area in skeletal muscle cells when larvae became excited (30 000 \times);
5. 处理组粘虫幼虫中毒抽搐期体壁肌细胞线粒体肿胀, 空泡化 Treated larvae showing swollen mitochondria, vacuolated skeletal muscle cells when larvae began to lose body fluid (30 000 \times);
6. 处理组粘虫幼虫中毒失水期体壁肌细胞线粒体肿胀, 嵴紊乱 Treated larvae showing swollen mitochondria and disordered cristae in skeletal muscle cells when larvae began to lose body fluid (30 000 \times).





